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Sample matrix effects in capillary electrophoresis

I. Basic considerations

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ABSTRACT

Serum samples contain high concentrations of proteins and ions which interfere in the analysis of small molecules such as theophylline. These effects are minimized by employing an electrophoresis buffer with high ionic strength. Although such buffers slow the speed of the analysis, they minimize the effects of proteins and ions, improve the separation and enable a larger volume of sample to be introduced into the capillary leading to enhanced signals. Therefore, serum samples can be diluted in weak buffers and analyzed directly rendering capillary zone electrophoresis an attractive technique in the clinical laboratories.

INTRODUCTION

The sample represents a small portion of the total capillary volume, yet it plays an important role in the overall separation and plate number. This is due to the nature of current conductance and the tendency of proteins to adsorb to the capillary walls. In general, plate number and peak height can be improved by using lower conducting conditions in the sample. This technique is known as stacking [1,2].

Analysis of serum or urine samples for endogenous substances and drugs presents special problems because of the high and variable content of proteins and ions. Although solvent extraction eliminates such effects, it is not suited for routine analysis in the clinical laboratories. To overcome these problems in high-performance liquid chromatography (HPLC), direct injection of diluted serum and acetonitrile deproteinization are quite commonly used for sample preparation [3–5]. These same techniques have been applied to capillary zone electrophoresis (CZE). It has been shown previously that both acetonitrile deproteinization and direct serum introduction are feasible as demonstrated by the analysis of iohexol by CZE [6]. Using micellar electrokinetic capillary electrophoresis, Thormann and co-workers [7,8] have shown that drug analysis by direct serum injection is also possible. The effect of proteins and different ions on the analysis of endogenous compounds and drugs in serum by CZE have not been extensively examined, mainly because the technique is relatively new. In this study, theophylline, a drug commonly analyzed in the clinical laboratory, was used as a model compound to study the effect of various sample parameters such as the presence of serum, proteins and ions, and the effects of sample volume on analysis of small molecules by CZE.

MATERIALS AND METHODS

Instrument

An automated capillary electrophoresis instrument (Beckman Instruments, Palo Alto, CA, USA) was set at 9 kV, 24°C and 280 nm. The capillary was 25 cm × 50 μm I.D. The elec-

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trophoresis buffer was in most experiments 100 m*M* borate adjusted to pH 8.8 with sodium carbonate. Samples were introduced by pressure injection for 5 s. The capillary was washed with NaOH (1 mmol/l, 0.7 min), phosphoric acid (50 mmol/l, 0.7 min) and electrophoresis buffer (1 min).

Standards

An aqueous solution containing 20 mg/l of theophylline and 20 mg/l of 8-chlorotheophylline (internal standard) (Sigma Chemicals, St. Louis, MO, USA) was used in these experiments. Peak height was used for quantitation. Serum and ions were added to this solution as described later on.

RESULTS AND DISCUSSION

The optimum conditions for separating theophylline from the internal standard 8-chlorotheophylline were investigated first. As expected, the separation was faster at higher voltages. A voltage of 9 kV was chosen for subsequent experiments. The ratio of theophylline to 8-chlorotheophylline seemed to be constant for peak height and migration time, indicating that the use of an internal standard can correct for changes in migration time and peak height. There was a good separation at pH 8.2 and 8.8, but the two compounds co-eluted at pH 10. Therefore, a pH of 8.8 was chosen for the following experiments. Changing the ionic concentration of the electrophoresis buffer between 50–200 mmol/l did not affect the peak height, but the high ionic strength increased the separation time. For the majority of the subsequent experiments a 100 mmol/l buffer concentration was used. Fig. 1A represents the separation of theophylline and 8-chlorotheophylline under these conditions.

After selecting the optimum conditions for separating theophylline and 8-chlorotheophylline, various factors present in the sample which might affect the separation by CZE such as the presence of proteins, ion concentration, and sample volume injected were studied.

The addition of moderate amounts of serum to aqueous standards of theophylline and 8-

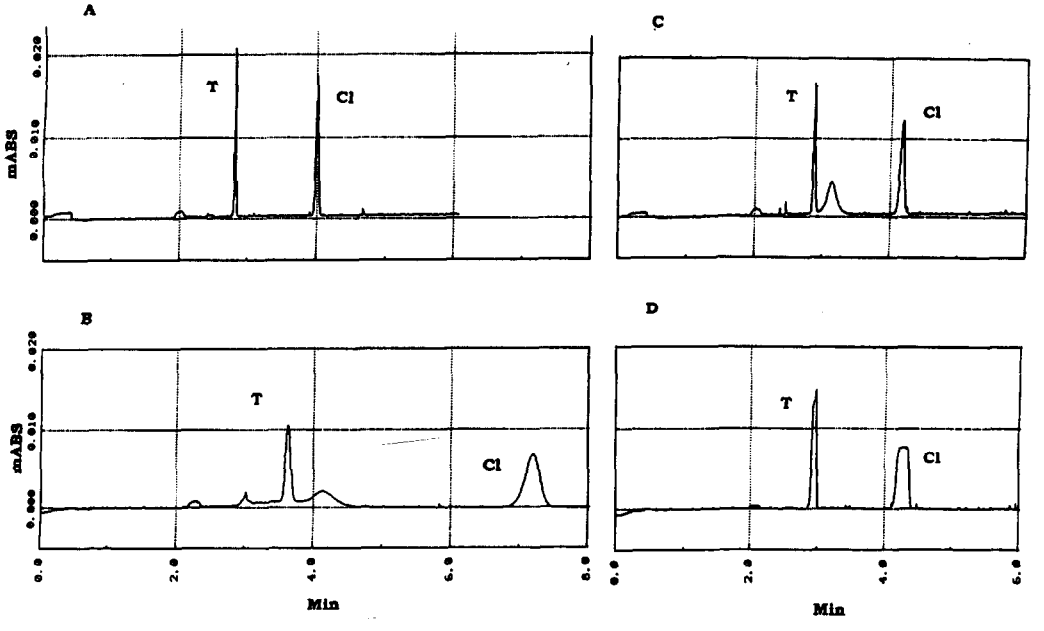

Fig. 1. Electropherogram for the separation of theophylline (T; 20 mg/l) and 8-chlorotheophylline (Cl; 20 mg/l) at 9 kV, 24°C, 280 nm, 100 m*M* borate buffer, pH 8.8 and 5 s pressure injection. (A) Sample dissolved in water; (B) as in A, but containing serum proteins at 3 g/l; (C) as in A, but containing albumin at 3 g/l; (D) sample dissolved in 100 m*M* borate buffer, pH 8.8 and introduced by pressure injection for 10 s.

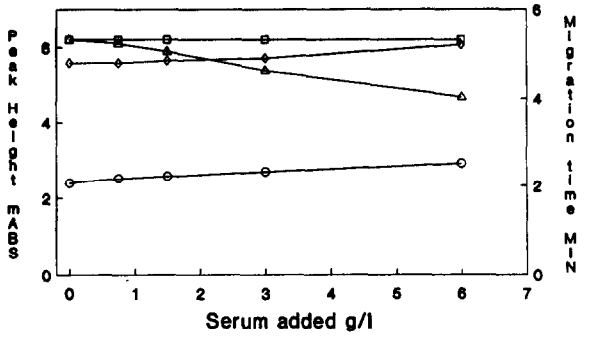

Fig. 2. Effect of different concentrations of serum added to aqueous solutions of theophylline with a 50 and 200 m*M* borate buffer, pH 8.8. Same separation conditions as in Fig. 1. □ = Peak height, 200 m*M*; △ = peak height, 50 m*M*; ◇ = migration time, 200 m*M*; ○ = migration time, 50 m*M*.

chlorotheophylline did not affect peak height or migration time appreciably; however, high concentrations (>3 g/l) affected peak height and increased the migration time (Fig. 1B). This effect is much more pronounced when an electrophoresis buffer of low ionic concentration is used (Fig. 2), and with high sample volumes. Albumin, the major serum protein, only caused a slight change in peak height at a high concentration (6 g/l) when using a low-ionic-strength buffer, and no effect was observed with a high-ionic-strength one (Figs. 3 and 1C). Addition of sodium chloride to the sample (Fig. 4), or borate ions decreased the peak height and increased the migration time as seen on the addition of serum.

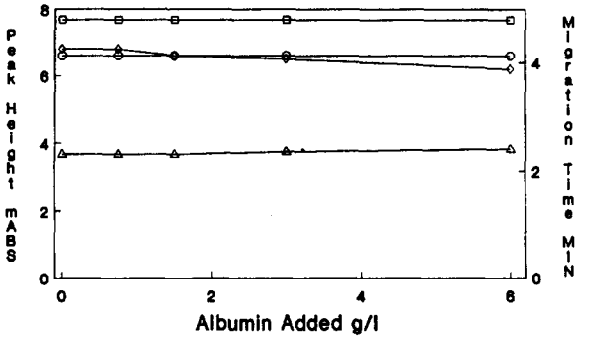

Fig. 3. Effect of different concentrations of albumin added to aqueous solutions of theophylline with a 50 and 200 m*M* borate buffer, pH 8.8. Same separation conditions as in Fig. 1. ◇ = Peak height, 50 m*M*; ○ = peak height, 200 m*M*; △ = migration time, 50 m*M*; □ = migration time, 200 m*M*.

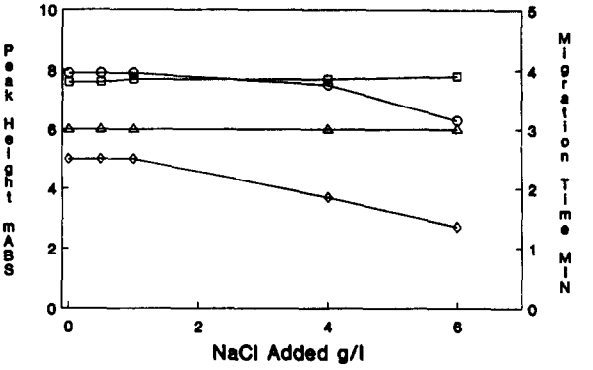

Fig. 4. Effect of different concentrations of NaCl added to aqueous solutions of theophylline and 8-chlorotheophylline. Same separation conditions as in Fig. 1. ○ = Theophylline peak height; △ = theophylline migration time; ◇ = 8-chlorotheophylline peak height; □ = 8-chlorotheophylline migration time.

At high concentrations of these ions, the peaks were wide (Fig. 1D) and sometimes split, especially when the sample volume was large (>10 s injection). Increasing the sample pH from 7.0 to 10.0 decreased the peak height, while the migration time remained constant.

Separations using electrophoresis buffers of low ionic strength are much more susceptible to various effects caused by high ionic concentrations of the sample (Fig. 5), especially when the sample volume is large (>5 s injection). On the other hand, separations with electrophoresis

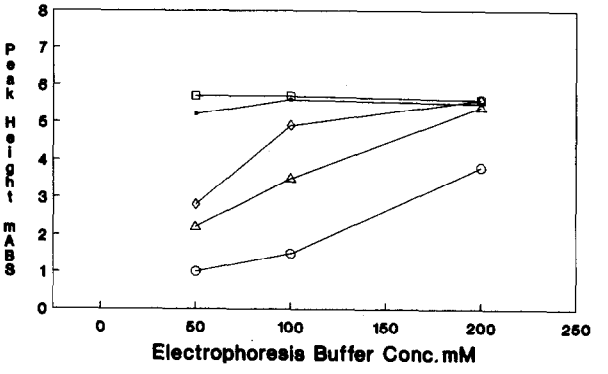

Fig. 5. Effect of different concentrations of borate ions added to aqueous solutions of theophylline. Electrophoresis buffers pH 8.8: 50, 100, 200 mM. Other separation conditions the same as in Fig. 1. Sample buffer concentration: □ = 0 m*M*; ● = 25 m*M*; ◇ = 50 m*M*; △ = 100 m*M*; ○ = 200 m*M*.

TABLE I

PRECISION OF THEOPHYLLINE PEAK HEIGHT AS A FUNCTION OF THE ELECTROPHORESIS BUFFER IONIC CONCENTRATION

Molarity (m*M*)	Relative standard deviation (%) ($n = 10$)	
	Theophylline	Theophylline/8-chlorotheophylline
25	2.2	1.3
100	1.9	1.3
200	0.9	0.9

buffers of high ionic strength are not as susceptible to these effects; however, the separations are slower. Even when assaying pure aqueous standards, the precision of the theophylline peak height tends to improve slightly by using high-ionic-strength buffers (Table I). Electrophoresis buffers of high ionic strength have the added advantages of giving better resolution and producing sharper peaks, *i.e.* higher plate numbers, especially for proteins [9,10]. These advantages are probably a consequence of the decrease in band diffusion.

Due to the small light path in CZE, the minimum detection level is limited; for this reason, the maximum amount of sample which can be introduced into the capillary in order to enhance the signal was investigated. Fig. 6 indicates that for serum samples, a high-ionic-strength buffer (300 m*M*) enabled a larger volume of sample to be introduced before the signal reached a plateau. The increased sample volume lead to an increase in sensitivity as reflected by the peak height (Fig. 6). Vinther and Soeberg [1], using a weak electrophoresis buffer (10 m*M* tricine), found that the plate number (N) decreased greatly with increasing sample size. In this work, using high-ionic-strength buffers, it was also observed that a larger sample volume decreased N, but to a lesser extent than that observed by Vinther and Soeberg. We have used this approach successfully for the determination of iohexol in serum [6].

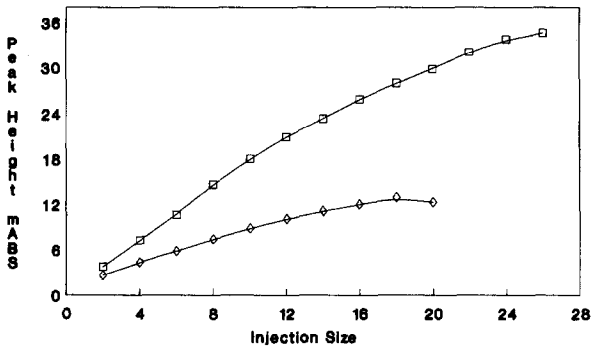

Fig. 6. Effect of the injection size (in seconds) of serum solutions of theophylline on peak height. Capillary: 25 cm × 50 μm. Electrophoresis buffers pH 8.8: 30 and 300 m*M*. Other separation conditions the same as in Fig. 1. □ = Buffer 300 m*M*; ◇ = buffer 30 m*M*.

CONCLUSIONS

Based on these results, it is evident that serum samples can be analyzed directly using a simple dilution in a low-ionic-strength buffer (10–20 times lower than that of the electrophoresis buffer). A 10–20-fold dilution of serum samples yields an acceptable peak shape, height and migration time, provided the molarity of the borate buffer is high enough (>200 mmol/l). Previously, it has been demonstrated using micellar electrokinetic capillary electrophoresis that serum can be directly injected without dilution [7,8]. This is possible because the micelles solubilize the serum proteins. Here we show that diluted serum samples can also be directly injected using CZE conditions.

Addition of standards directly into serum rather than into aqueous solutions will keep the migration time and peak height very close to the unknown samples [6,8]. It is, however, important to remove the small amounts of proteins

which adsorb to the capillary walls with thorough washings between samples. It is recommended to use high-ionic-strength buffers when analyzing samples derived from serum or urine even though these buffers slow the analysis time. The analysis time can be shortened by using a short capillary. The high strength buffers allow a larger sample volume to be introduced into the capillary without a significant sacrifice in plate number leading to an enhanced detector signal.

Since protein removal is not necessary, CZE becomes an attractive technique for analysis in clinical laboratories, provided the compound of interest produces an adequate detector signal. Traditional solvent extraction, of course, eliminates the problem of proteins and ions present in the sample with an enhancement of the detection due to sample concentration. However, it requires several additional steps increasing the analysis time.

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